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Efficacy of chloroquine + sulfadoxine–pyrimethamine, mefloquine + artesunate and artemether + lumefantrine combination therapies to treat *Plasmodium falciparum* malaria in the Chittagong Hill Tracts, Bangladesh

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Summary Bangladesh faces growing levels of *Plasmodium falciparum* resistance to chloroquine (CQ) and sulfadoxine–pyrimethamine (SP). Alternative antimalarial therapies, particularly combination regimens, need to be considered. Therefore, the efficacy of three antimalarial combination therapies was assessed in Chittagong Hill Tracts. A total of 364 *P. falciparum* patients were recruited and randomly assigned to either CQ + SP, mefloquine + artesunate (MQ + AS) or lumefantrine + artemether (Coartem©). Results showed that CQ + SP therapy was less effective than the two artemisinin-based combination therapies. The day 42 PCR-corrected efficacy rate was 62.4% for CQ + SP, 100% for MQ + AS and 97.1% for Coartem. Failures occurred at a shorter interval after CQ + SP treatment than after Coartem. The artemisinin-based therapies effectively prevented development of

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gametocytes, whereas CQ+SP did not. All three therapies were well tolerated, although reports of mild complaints during treatment appeared higher with MQ+AS. We conclude that CQ+SP is not a viable option for replacing CQ monotherapy as first-line *P. falciparum* treatment in this area of Bangladesh. A change to artemisinin-based combination therapy is recommended. Both Coartem and MQ+AS appear to be good options, effective in curing *P. falciparum* malaria and in preventing recrudescences following treatment.

1. Introduction

Multidrug resistance of *Plasmodium falciparum* parasites has developed in Asia earlier than in other malarious areas around the world. As early as 1957, chloroquine (CQ) resistance appeared, whilst sulfadoxine–pyrimethamine (SP) resistance first emerged in 1967, both at the Thai–Cambodian border. Since then, it has been described in all Asian countries. Drug resistance is enhanced with patterns of drug availability and drug use (Hastings, 2001; Wongsrichanalai et al., 2002). Generally across Asia, pharmacy shops are found at every street corner and people have the tendency to try to cure an infection without proper diagnosis, by simultaneous use of a cocktail of various medicines. In Bangladesh, the national drug policy is quite strict and the list of medicines registered for import is limited. However, these restrictions, together with clear case definitions and treatment guidelines for malaria, have not been able to block the spread of resistance to the country's malaria-endemic areas bordering India and Myanmar (Rahman et al., 1996, 2001).

Changes in national treatment policies of Asian countries in response to rising levels of resistance have been slow at first, but currently the majority of countries in this region have made a switch from CQ to artemisinin-based combination therapies (ACT), such as artesunate combined with mefloquine, amodiaquine or SP, Coartem or a novel combination called CV8 (Bosman, 2004; Giao et al., 2004). Bangladesh lingered over a change in treatment protocols, but recently the Ministry of Health and Family Welfare (MoHFW) decided on implementation of artemether–lumefantrine (Coartem©) as a new national policy to treat uncomplicated falciparum malaria in the future, if the required funding becomes available (JICPD, 2004).

The change in national malaria treatment protocols were planned but not yet decided at the time of this trial. Further insight into the pros and cons of different therapies was needed. ACTs are the preferred option because of their high efficacy, rapid cure and capacity to reduce gametocyte development (WHO, 2001); however, their high cost remains a barrier to implementation. Taking these considerations into account, the MoHFW of Bangladesh, the WHO and Médecins Sans Frontières (MSF) conducted this efficacy trial on three drug combination therapies, namely artemether and lumefantrine (Coartem), mefloquine+artesunate (MQ+AS) and CQ+SP, which could possibly be introduced as alternative antimalarial protocols in the future.

2. Patients and methods

2.1. Study location

Located in the eastern part of Bangladesh, the Chittagong Hill Tracts (CHT) encompasses mountainous, forested land with an elevated yearly rainfall, characteristic for high malaria transmission in Asia. With one case per five inhabitants annually (MoHFW, 2002; malaria cases, clinical and confirmed, reported per district), it is one of the areas of highest endemicity in Southeast Asia. The rainy season is from May to October. The ethnic composition of the population is Chakma, Marma and Tripura tribal groups and Bengali settlers. Health services in the rural areas of CHT were disrupted during preceding periods of instability. These were still understaffed and insufficiently supplied when MSF started a basic healthcare project in 1998, with two outpatient clinics situated in Khagrachari Hill District near the Indian border. The statistics from the MSF clinics confirm the scale of malaria as a threat to public health. More than 30% of all patients are ill from malaria, ~85% *P. falciparum* monoinfection or mixed infections, 15% *P. vivax* and 1% *P. malariae* (MSF-Holland data 2003). Malaria incidence shows a clear seasonal pattern, and young and old as well as males and females are affected at similar ratios.

2.2. Study design

The study design was that of an open-label, randomised efficacy trial, with three different treatment arms. Treatment allocation was stratified by age group, i.e.: (1) 1 year to below 5 years of age; (2) from 5 years to 14 years old; and (3) 15 years and older. The sample size of 120 patients per treatment group was based on an estimated 75% efficacy of CQ+SP and 90–100% for the two ACTs, aiming to detect a difference in cure rates of 15%, with 80% power, 5% significance level and anticipating a default rate of 20% (Campbell and Machin, 2000). The study procedures follow WHO guidelines for low transmission zones (WHO, 1996, 2003).

2.3. Inclusion and exclusion criteria

Patients recruited were at least 1 year old, with a *P. falciparum* monoinfection of 1000–100 000 asexual malaria parasites per μl and (a history of) fever. They were only included after written informed consent was given by themselves or their caretakers. Excluded were pregnant women, patients with severe anaemia (haemoglobin (Hgb) <6 g/dl), signs of severe malaria or another febrile or serious disease requiring treatment.

2.4. Treatment

Treatment was randomly assigned and given in accordance with the patient's body weight: (1) 10 mg/kg CQ and 25 mg S – 1.25 mg P per kg single dose on day 0, 10 mg/kg CQ on day 1 and 5 mg/kg on day 2; (2) 15 mg/kg MQ+4 mg/kg AS on day 0, 10 mg/kg MQ+4 mg/kg AS on day 1 and 4 mg/kg AS on day 2; (3) artemether–lumefantrine (Coartem), two doses per day over 3 days according to weight, with a minimum of 6 h to a maximum of 12 h between daily doses. Randomisation was done in blocks of 30 and stratified per age group by drawing a card from a box assigned to the respective age group, initially containing 30 cards (10 cards per treatment). When 30 cards were finished, the box was replenished with new ones. Patients in all treatment groups came back on days 1 and 2 for observed daily treatment. In addition to this, Coartem patients took one evening dose per day at their homes in front of an observer (neighbour or village volunteer). Each dose of Coartem was taken with 250 ml of sweetened milk.

Patients who failed treatment were re-treated with oral quinine 30 mg/kg/day for 7 days. Patients with *P. vivax* or *P. malaria* infections were given CQ (25 mg/kg over 3 days) but were kept in the study none the less. All drugs were purchased from IDA, The Netherlands, except Coartem, which was from Novartis, Bangladesh.

2.5. Clinical procedures

Treatment allocation was done by drawing a card from a box (one for each age group) containing three types of cards coding for treatments. All patients received medication under observation; they returned on days 1, 2, 3, 7, 14, 21, 28, 35 and 42 and any other day when feeling ill. Patients were traced at home when not returning for follow-up. At each visit, clinical signs indicating malaria disease or possible side effects during treatment were recorded. Any other disease was treated accordingly. To compensate for travel costs and time lost, patients received a small fee at each visit. The efficacy of treatment was evaluated by the parasitological and clinical response and was classified in accordance with WHO guidelines for low transmission areas (WHO, 1996, 2003) as: (1) early treatment failure (ETF) defined as a case exhibiting signs of severe malaria within 3 days after treatment, or showing a rise in parasitaemia above the admission level on day 2, or a parasitaemia of ≥25% of that on admission, or parasites in the blood in the presence of fever on day 3; (2) late clinical failure (LCF) in case of danger signs, or parasites in the blood in the presence of fever at any day between 4 and 42; (3) late parasitological failure (LPF) when parasites where present but axillary temperature was below 37.5 °C on any day from day 7 to day 42; and (4) adequate clinical and parasitological response (ACPR) when parasites were absent on day 42 irrespective of axillary temperature without previously meeting any of the criteria of ETF, LCF or LPF.

2.6. Laboratory procedures

Blood slides were stained with a 5% solution Giemsa for 25 min. The density of *P. falciparum* trophozoites was assessed by parasite/white blood cell (WBC) count, assuming a standard density of 8000 WBC/μl (WHO, 1991). On days 0 and 28, the blood Hgb value was checked with a Haemocue® digital meter. At day 0 and the day of treatment failure, blood samples on filter paper were collected for PCR analysis. The distinction between recrudescences and reinfections was performed at the Shoklo Malaria Research Unit, Thailand, based on a previously described protocol (Brockman et al., 1999). Briefly, the *P. falciparum msp-1, msp-2* and *glurp* gene loci of pre- and post-treatment sample pairs were compared to determine whether the genotype before and after treatment was identical, indicating a recrudescence. In a previous study

in the same clinics (van den Broek et al., 2004), it was shown that the genetic variation in the parasite population based on the three gene loci examined is sufficient for this assumption (average number of genotypes per infection of 1.3; probability to detect the same genotype pre and post treatment by chance alone <0.05). The outcome of treatment with PCR correction was based on the number of true recrudescences, excluding cases of novel infections or indeterminate PCR from analysis.

2.7. Ethical approval and quality control

The study protocol was approved by the MoHFW and received ethical clearance from the Bangladesh Medical Research Council. In addition, permission from the District Council Chairman of the Local Government *Parishad* was taken and local health authorities were informed. The Medical Department of MSF also reviewed the study proposal. All drugs used in this study had been produced under the principles of Good Manufacturing Practice. Microscopy results were crosschecked by external laboratories for 10% of slides. Data were double-entered and analysed with SPSS (version 10.05; SPSS Inc., Chicago, IL, USA) and Epi Info (6.04; CDC, Atlanta, GA, USA), using χ^2 test and Fisher exact tests for categorical comparisons and analysis of variance (ANOVA) for continuous variables.

3. Results

During the period May to September 2003, a total of 364 *P. falciparum* patients were recruited for study and assigned to one of the three therapies. The baseline characteristics of the patients were comparable among treatment groups (Table 1; $P > 0.05$ for all comparisons). Twenty patients did not complete follow-up for different reasons: eight were lost to follow-up, three vomited repeatedly (two CQ+SP, one MQ+AS), five were misclassified as treatment failures and re-treated (all on CQ+SP), and four received malaria treatment elsewhere.

During the 6 weeks of follow-up, 35 patients had a *P. vivax* infection (3 mixed with *P. falciparum* at treatment failure). The *P. vivax* infections were unequally distributed over the treatment groups: 25 were in the Coartem group, 6 on MQ+AS and 4 on CQ+SP ($P < 0.0001$). They appeared late in follow-up (median 35 days, range 21–42 days). One *P. malariae* infection was observed (Coartem group, day 35).

At the end of the study, 300 blood slides were crosschecked in Khagrachari Sadar District Hospital, and 40 slides at Dhaka Central Malaria Reference Laboratory. The proportion of disagreement was 4.4% (15/340); disagreement slides were reviewed and in two cases resulted in reclassification of LPF to ACPR.

3.1. Efficacy of the three combination therapies

The number of patients with a recurrent *P. falciparum* parasitaemia during follow-up reached 58 for CQ+SP (52%), 9 for MQ+AS (8%) and 20 for Coartem (17%). The proportion for CQ+SP was significantly higher than for the other two therapies ($P < 0.0001$), and it was also higher for Coartem than for MQ+AS ($P = 0.039$). Patients came back positive at a shorter time interval after CQ+SP treatment than after the two ACTs ($P < 0.0001$) (Figure 1).

PCR analysis was done on 83 samples of 87 recurrent cases (3 were ETF and therefore considered recrudescences *per se*; 1 sample was missing); 32

Table 1 Baseline characteristics of patients per treatment group

	CQ+SP	MQ+AS	Coartem
No. of patients (% of total)	122 (33.5)	121 (33.2)	121 (33.2)
Sex: number of F/M (% F)	58/64 (47.5)	51/70 (42.1)	58/63 (47.9)
Age (years)[a]	15.0±0.93 (1.3–50)	14.2±1.10 (1.2–80)	15.7±1.01 (2.5–66)
No. under 5 year olds	16 (13.1)	22 (18.2)	17 (14.0)
5–14 years	47 (38.5)	50 (41.3)	48 (39.7)
≥15 years	59 (48.4)	49 (40.5)	56 (46.3)
Weight (kg)[a]	32.7±1.4 (7.5–75)	30.4±1.5 (7.5–80)	32.6±1.3 (10–64)
Haemoglobin (g/dl)[a]	11.5±0.2 (5.4–16.4)	11.6±0.19 (7.2–17.2)	11.7±0.17 (5.6–16.3)
Temperature (°C)[a]	37.5±0.1 (35.5–40.1)	37.7±0.1 (35.8–40.1)	37.7±0.1 (35.1–40.2)
Parasite density (per μl)[b]	12 016 (1120–96 600)	14 271 (1006–97 760)	11 814 (1220–82 560)

CQ: chloroquine; SP: sulfadoxine–pyrimethamine; MQ: mefloquine; AS: artesunate.
[a] The values are given as mean ± SD and range (min.–max. value).
[b] Parasite density given as geometric mean and range.

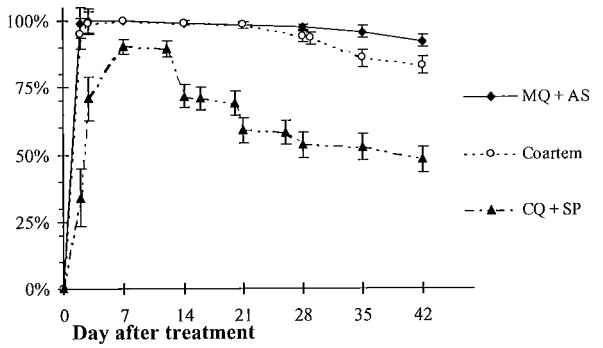

Figure 1 Proportion of patients parasite-free during follow-up.

recrudescences and 32 novel infections were identified. Reinfections numbered 16 in the CQ+SP group, 5 in the MQ+AS group and 11 in the Coartem group. Some paired samples could not properly be compared owing to inability to identify a 3-locus genotype ($n=16$) or detection of multiple alleles at one or more gene loci ($n=3$). The 52 novel, indeterminate and missing cases were all excluded from the final analysis.

PCR-corrected efficacy at day 42 was 62.4% (95% CI 51.2–72.6), 100% (95% CI 96.5–100) and 97.1% (95% CI 91.6–99.4) for CQ+SP, MQ+AS and Coartem, respectively (Table 2). The PCR-corrected outcome of the CQ+SP group was significantly different from the ACT groups ($P<0.0001$), whereas the difference between the two ACTs was not significant ($P=0.12$).

Comparison of the three age groups used for treatment randomisation showed that treatment efficacy was relatively low in the group of children under 5 years of age compared with the other two groups, whilst the latter were similar (children <5 years vs. those over 5 years: 53.3% for CQ+SP, 90.1% for MQ+AS and 94.1% for Coartem vs. 63.5%, 97.8% and 92.2%, respectively). However, the group of under 5 year olds appeared to be too small in number to allow for a good comparison, therefore the differences were not significant ($P>0.05$).

3.2. Effect on gametocytes

At the day of admission, only 2% of patients had gametocytes. This increased at days 2 and 3, more markedly after CQ+SP compared with MQ+AS and Coartem (*P*-values). During the follow-up period of 42 days, a total of 46% of patients in the CQ+SP group had gametocytes at one or more visits, whereas it was only 0.8% and 2.5% of patients treated with MQ+AS and Coartem, respectively (Figure 2).

3.3. Adverse events

No severe adverse clinical events were observed. Mild adverse events during the 3 days of treatment were headache, vomiting, nausea and dizziness. The frequency of these potential treatment-related complaints was generally higher after MQ+AS treatment than after Coartem ($P<0.05$) (Figure 3). After CQ+SP treatment, complaints were of intermediate frequency, but vomiting occurred more in this group. Other complaints were anorexia, skin itching and deafness with CQ+SP, sleeplessness, anorexia, skin itching/rash, epigastric pain and

Table 2 Treatment efficacy of the three therapies, with PCR adjustment, after 28 days (standard cut-off) and 42 days endpoint (extended)

	CQ+SP			MQ+AS			Coartem		
	n	%	95% CI	*n*	%	95% CI	*n*	%	95% CI
Day 28[a]	91			114			113		
ACPR	61	67.0	56.3–76.3	113	99.1	94.5–100	112	99.1	95.2–100
ETF	3	3.3	0.9–10.0	0	0	0–3.2	0	0	0–3.2
LCF	4	4.4	1.2–10.9	0	0	0–3.2	0	0	0–3.2
LPF	23	25.3	16.7–35.5	1	0.9	0–4.8	1	0.9	0–4.8
Day 42[a]	85			105			102		
ACPR	53	62.4	51.2–72.6	105	100	96.5–100	99	97.1	91.6–99.4
ETF	3	3.5	0.7–10	0	0	0–3.6	0	0	0–3.6
LCF	5	5.9	1.9–13.2	0	0	0–3.6	1	1	0–5.3
LPF	24	28.2	19.0–39.0	0	0	0–3.6	2	2.0	0.2–6.9
Total failures	32	37.6	27.4–48.8	0	0	0–3.6	3	2.9	0.6–8.4

CQ: chloroquine; SP: sulfadoxine–pyrimethamine; MQ: mefloquine; AS: artesunate; ACPR: adequate clinical parasitological response; ETF: early treatment failure; LCF: late clinical failure; LPF: late parasitological failure.

[a] Novel infections, and missing or undetermined PCR results, were excluded from analyses.

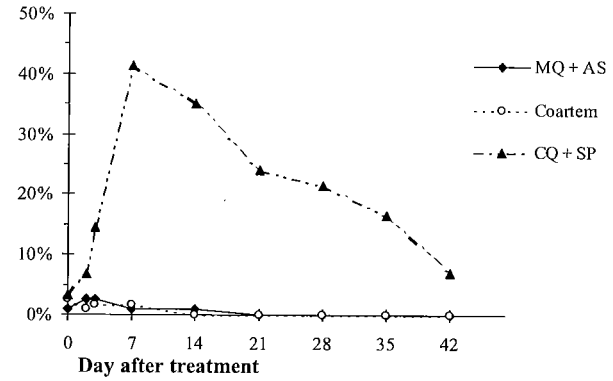

Figure 2 Proportion of patients with gametocytes in their blood during follow-up.

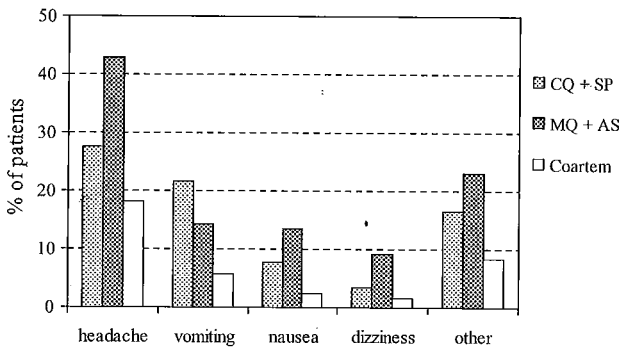

Figure 3 Reported symptoms during 3 days of treatment.

excessive sweating with MQ + AS, and blurred vision and anorexia with Coartem.

4. Discussion

This study is the first comparative trial of the efficacy of three combination therapies including Coartem and MQ + AS for uncomplicated *P. falciparum* malaria in Bangladesh. The results suggest that the combination CQ + SP is not a viable option for treatment of falciparum malaria in this area, whereas the two ACTs, MQ + AS and Coartem, both appear to be very effective to clear parasitaemia and to prevent recrudescent infections in falciparum malaria patients of all age groups.

4.1. CQ + SP

The CQ + SP treatment resulted in 38% of failures at 6 weeks. The low efficacy of the single drugs CQ and SP has been found in earlier studies in Bangladesh of in vivo and in vitro efficacy as well as confirmed by high prevalence of *pfmdr1* and *pfcrt* mutations related to CQ resistance (Noedl et al., 2003; Rahman et al., 2001; van den Broek et al., 2004). In general, it can be expected that in areas with high levels of *P. falciparum* resistance to CQ and moderate resistance to SP, the combination of these two does not achieve better cure rates than SP alone (WHO, 2001). In Bangladesh, CQ + SP was considered by the MoHFW as a potentially viable and affordable first-line treatment to replace CQ. However, in addition to our findings, two recently completed studies in adjacent areas in CHT also revealed limited efficacy of the combination of CQ + SP (Rahman et al., 2004). In all three studies, the level of treatment failures well exceeds the 25% that is the tolerable limit defined by the WHO, all of which suggests that these options should no longer be pursued in Bangladesh.

4.2. Artemisinin-based combination therapy

Both ACTs appear to be more effective options than CQ + SP. MQ + AS showed zero failure rate at day 42 and Coartem 2.9%. The difference between the two ACTs was not significant. However, the patient group treated with Coartem experienced more cases of *P. falciparum* during follow-up than the MQ + AS group, and more *P. vivax* infections were also found after Coartem treatment. This might be related to the prophylactic effect due to the longer half-life of MQ (ranging from 15 days to 33 days) (Winstanley, 2001) compared with the half-life of lumefantrine (3–6 days; Bloland, 2001). At the same time, however, this characteristic of MQ is a reason for concern when administered together with the very short-acting AS because residual subtherapeutic doses may favour the selection of resistant parasites, especially in areas of intense transmission. In Bangladesh, low-grade MQ resistance may be present already, as shown in in vivo and in vitro studies (Noedl et al., 2003; Rahman et al., 1998). Nevertheless, MQ + AS has shown high efficacy in Myanmar Rakhine State, just across the border from CHT (Smithuis et al., 2004). Also Thailand, where MQ + AS has now been in use for 10 years, has shown a very promising prospective: instead of further invasion of MQ resistance, the initial levels of resistance were actually brought down by using the two drugs strictly in combination (Nosten et al., 2000; Price et al., 1996). In Bangladesh, MQ is not regularly used because of limited availability. AS is rarely ever used because it is not officially registered.

Our results confirm the known effect of artemisinins to block the development of new gametocytes. This effect of ACT has potential

implications for the transmission of *P. falciparum* malaria. CQ+SP therapy, especially SP, leaves gametocyte development unaffected (Mendez et al., 2002; von Seidlein et al., 2001).

Clinical complaints of patients during the 3 days of treatment were somewhat higher with MQ+AS therapy than with Coartem. Other studies have shown that side effects such as anorexia, nausea, vomiting, dizziness and sleeping disorders can occur after administration of MQ, but that these are reduced when the 25 mg/kg dose is split and given over 2 days, and even more so when combined with AS (Smithuis et al., 2004; ter Kuile et al., 1995).

4.3. Limitations of the study

Coartem is very effective in treating for *P. falciparum* malaria, but requires a six-dose regimen over 3 days (Lefevre et al., 2001; van Vugt et al., 1999; von Seidlein et al., 1998). In our study, Coartem treatment was partly observed by the study team (morning dose) and partly by a village volunteer (evening dose), as the clinic set-up and security situation prevented us working in the evening or night. Although maximum efforts were made to enhance compliance, there might have been a proportion of patients who did not take the treatment as prescribed. Non-compliance can be expected to be higher in the 'real life' situation. However, even without observing the evening intake, the failure rate was found to be extremely low.

Adherence to treatment can further be improved with proper information to all patients and the use of blister packages per age group (Fogg et al., 2004). A further difficulty of Coartem is that absorption of lumefantrine can be severely reduced in fasting patients. Food intake, especially fatty food, is needed for proper uptake of this drug (Ezzet et al., 2000) and this requirement might not easily be fulfilled by patients living in this area of Bangladesh. In our study, we supplied patients with a package of sweetened milk for each dose to standardise this aspect of drug uptake.

The over-distribution of *P. vivax* infections in the Coartem group cannot be explained as an effect of treatment; all three treatments tested here are considered effective to treat *P. vivax*. It may suggest a difference in distribution of infections in the three treatment groups. Even if the patients in the Coartem group were somehow more likely to be infected (e.g. due to living circumstances), it still produced good results and is therefore not critical to the findings and conclusions.

4.4. Considerations for national treatment policy

Both Coartem and MQ+AS have proven to be very effective treatment regimens. MQ+AS might be preferred over Coartem, but for MQ+AS there needs to be access to AS in Bangladesh, which requires official registration in the country. Coartem is registered with the National Drug Administration in Bangladesh but is not (yet) released for infants below 10 kg or for pregnant women. In the MSF clinics, Coartem treatment has been implemented on a try-out basis since August 2003. In a small survey, 93 out of 100 patients apparently completed treatment and only one of the non-compliers became positive again at day 14 (MSF 2003, unpublished data).

Fortunately, the population at risk of malaria is limited to the hill districts on the northern and eastern border and concerns only 10% of Bangladesh's huge population. The number of falciparum malaria patients at present appears to increase from one year to the next in CHT and the seasonal peak in numbers of (severely) ill patients and deaths from malaria is highly worrying.

Diagnostic facilities for malaria need to be further developed in order to move away from clinical diagnosis (Faiz et al., 2002). Upgrading of the laboratory facilities will need extra resources and time for training and implementation. Use of rapid tests for malaria diagnosis could be a good short-term option.

Conflicts of interest statement

The authors have no conflicts of interest regarding the work reported in this paper.

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